

ORIGINAL RESEARCH ARTICLE

# NMDA receptor antagonists ketamine and PCP have direct effects on the dopamine D<sub>2</sub> and serotonin 5-HT<sub>2</sub> receptors—implications for models of schizophrenia

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**Ketamine and PCP are commonly used as selective NMDA receptor antagonists to model the putative hypoglutamate state of schizophrenia and to test new antipsychotics. Recent findings question the NMDA receptor selectivity of these agents. To examine this further, we measured the affinity of ketamine and PCP for the high-affinity states of the dopamine D<sub>2</sub> and serotonin 5-HT<sub>2</sub> receptor and found that ketamine shows very similar affinity at the NMDA receptor and D<sub>2</sub> sites with a slightly lower affinity for 5-HT<sub>2</sub> (0.5 μM, 0.5 μM and 15 μM respectively), while PCP shows similar affinity for the NMDA and 5-HT<sub>2</sub> sites, with a slightly lower affinity for the D<sub>2</sub> site (2 μM, 5 μM and 37 μM respectively). Further, ketamine and PCP in clinically relevant doses caused a significant increase in the incorporation of [<sup>35</sup>S]GTP-γ-S binding in CHO-cells expressing D<sub>2</sub> receptors, which was prevented by raclopride, suggesting a partial agonist effect at the D<sub>2</sub> receptor. Thus, ketamine and PCP may not produce a selective hypoglutamate state, but more likely produce a non-selective multi-system neurochemical perturbation via direct and indirect effects. These findings confound the inferences one can draw from the ketamine/PCP models of schizophrenia.**

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## Introduction

Studies of patients with schizophrenia and of the mechanism of action of antipsychotics strongly support the view that there is a hyperactive dysregulation of the dopamine system in schizophrenia (evidence reviewed in references).<sup>1–3</sup> It has been proposed that this dopaminergic dysregulation is secondary to a primary glutamate deficiency in schizophrenia—the ‘hypoglutamate’ hypothesis of schizophrenia.<sup>4–6</sup> Direct evidence for a deficiency in the glutamate system in schizophrenia, or for the efficacy of glutamate agonist compounds is limited and conflicting.<sup>7</sup> However, the hypothesis remains attractive on the basis of animal models wherein challenges with non-competitive NMDA receptor antagonists, ketamine and PCP (thought to recapitulate the primary hypoglutamate state of schizophrenia), lead to the production of hyperlocomotion and sensorimotor gating deficits thought to be the underlying clinical abnormalities of schizophrenia.<sup>8</sup> Further it has been suggested that challenges with ketamine and PCP, since they model the puta-

tively primary hypoglutamate state, are superior to the more conventional apomorphine or amphetamine models, which are thought to model a secondary hyperdopaminergic state.<sup>9</sup> Thus, it is suggested that these challenges provide a better model for the illness and constitute better models for identifying ‘atypical’ antipsychotics.

There is little question that the administration of ketamine and PCP leads to diverse changes in the dopamine, serotonin and other monoamine systems.<sup>10,11</sup> According to the hypoglutamate hypothesis, these dopamine and serotonin effects are secondary to a primary hypoglutamate effect of ketamine and PCP.<sup>5</sup> However, a number of intriguing findings suggest that ketamine and PCP may not be selective models of a primary hypoglutamatergic state, but may in fact have multiple actions. For example, a recent study by Tsukada *et al* found that ketamine administration resulted in a decrease in selective [<sup>11</sup>C]-raclopride binding, without a concomitant increase in dopamine levels, raising the possibility that ketamine binds directly to D<sub>2</sub> receptors.<sup>12</sup> Similar concerns have also been raised about PCP, which has been shown to demonstrate direct protective effects at the serotonin 5-HT<sub>2</sub> receptor, suggesting direct or allosteric binding to the 5-HT<sub>2</sub> receptor.<sup>13</sup> Further, it has been suggested that ketamine and PCP may enhance endogenous levels of dopamine and serotonin via a direct effect on monoamine reuptake trans-

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porters,<sup>14</sup> a finding recently confirmed using cloned monoamine-reuptake receptors at clinically relevant doses.<sup>15</sup>

Since the hypoglutamate model, as established by ketamine and PCP challenges, continues to be a popular model for the study of schizophrenia, the objective of the present study was to investigate the direct effects of ketamine and PCP on the dopamine D<sub>2</sub> and the serotonin 5-HT<sub>2</sub> receptor, particularly on the high-affinity states of these receptors as these are the functionally relevant states.<sup>16</sup> Once we observed that ketamine and PCP showed a relevant affinity for these receptors in clinical concentrations, and distinguished between the high- and low- affinity states of these receptors, we examined the effect of ketamine and PCP on [<sup>35</sup>S]GTP- $\gamma$ -S to examine if they functioned as partial agonists on these receptors.

## Methods

### Tissue

Frozen rat brains were purchased (Pel-Freez Biologicals, Rogers, AR, USA) and stored at -70°C until used. The brain was partly thawed and the striata removed. The striata (or frontal cortex for serotonin 5HT<sub>2A</sub> receptors) were homogenized with a Polytron (PT-10 probe, Brinkmann Instruments, Westbury, NY, USA; setting 5 out of a maximum of 10) for 5 s in buffer (4 mg frozen tissue per ml buffer). The buffer contained 50 mM Tris-HCl (pH 7.4 at 20°C), 1 mM EDTA, 5 mM KCl, 1.5 mM CaCl<sub>2</sub>, 4 mM MgCl<sub>2</sub> and either 10 mM or 120 mM NaCl (as indicated in particular experiments). The homogenate was not washed or centrifuged because our previous work found that more than 30% of the receptors can be lost by these procedures.<sup>17</sup> For affinity studies involving cloned receptors we used the cloned dopamine D<sub>2</sub> long (human) receptor expressed in Chinese Hamster Ovary cell line (CHO), grown and treated as described previously.<sup>18</sup> The cells were suspended at 200  $\mu$ g protein ml<sup>-1</sup> and the suspension was homogenized for 5 s (Polytron, setting 5). The rest of the procedures for cloned tissue were similar to that for brain tissue.

### Radiolabeled ligands and reagents

The affinities of the drugs for the various receptors were measured by competition against the following ligands. For dopamine D<sub>2</sub> receptors [<sup>3</sup>H]raclopride (60–80 Ci mmol<sup>-1</sup>; final concentration of 2 nM in the incubation tube; non-specific binding defined in the presence of 10  $\mu$ M S-sulpiride) and [<sup>3</sup>H]dopamine were used (50 Ci mmol<sup>-1</sup>, final concentration of 0.2 nM in the incubation tube; non-specific binding defined in the presence of 200 nM haloperidol). For serotonin 5-HT<sub>2</sub> receptors [<sup>3</sup>H]ketanserin was used (76–81 Ci mmol<sup>-1</sup>; final concentration of 0.5–1 nM; non-specific binding defined in the presence of 1  $\mu$ M (+)-butaclamol). For the NMDA receptor [<sup>3</sup>H]MK801 was used (23 Ci mmol<sup>-1</sup>; final concentration of 6 nM in the incubation tube; non-specific binding defined in the presence of 10  $\mu$ M dextrorphan). For experiments

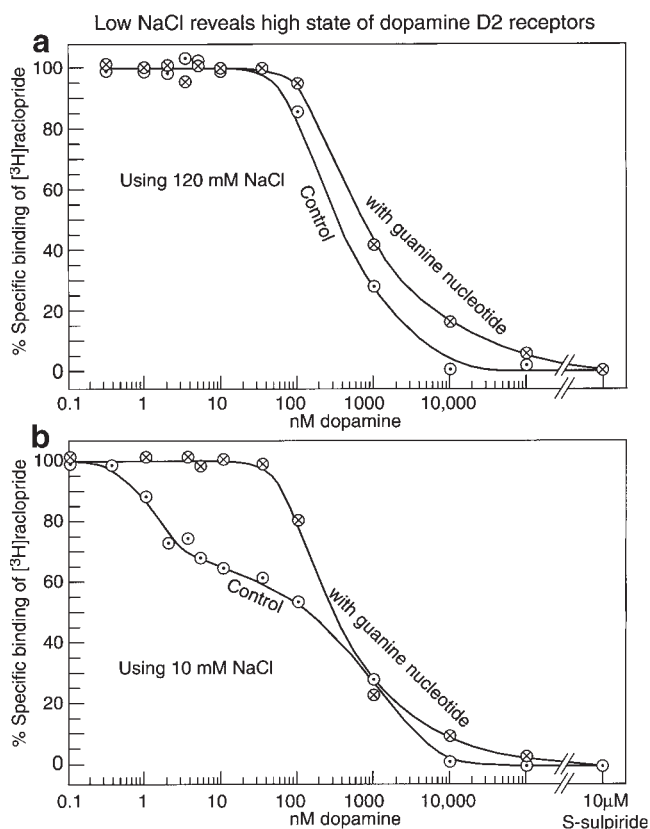
examining ligand-induced GTP incorporation [<sup>35</sup>S]GTP- $\gamma$ -S was used (1250 Ci mmol<sup>-1</sup>; final concentration of 0.2 nM in the incubation tube). Radical components were purchased from NEN Life Science Products, Boston, MA, USA. S-ketamine is known to have a higher affinity for the NMDA receptor than R-ketamine. Since we had access to S-ketamine (provided courtesy of Prof HA Adams) but not R-ketamine, we examined the effects of S-ketamine vs the racemic mixture on the dopamine D<sub>2</sub> and NMDA receptors.

### Affinity determinations

The competition between a drug and a [<sup>3</sup>H]ligand for binding at the receptors was done as follows. Each incubation tube (12  $\times$  75 mm glass) received the following in order: 0.5 ml buffer with or without appropriate concentration of the test drug, 0.5 ml [<sup>3</sup>H]ligand and finally 0.5 ml of tissue homogenate. The tubes containing a total volume of 1.5 ml were incubated for 2 h at room temperature (20°C) after which the incubates were filtered using a 12-well cell harvester (Titertek, Skatron, Lier, Norway) and buffer-pres soaked glass fiber filter mats (No. 7034, Skatron, Sterling, VA, USA). The filter mat was rinsed with buffer for 15 s (7.5 ml buffer). The filters were pushed out and placed in scintillation minivials (Packard Instruments, Chicago, IL, USA). The minivials received 4 ml each of scintillant (Ready Solve, Beckman Co, CA, USA) and were monitored 6 h later for scintillation in a Packard 4660 scintillation spectrometer at 55% efficiency. The experiments were carried out in duplicate and the affinity reported was based on the mean of 3–5 separate runs, unless otherwise stated the results are reported as mean  $\pm$  standard error of mean (SEM).

The high-affinity states of G-linked receptors are sensitive to experimental conditions. As a result there is a wide range of values for the affinity of dopamine at the D<sub>2</sub>High site (the high-affinity state of this receptor), 2 nM<sup>19</sup> to 75 nM.<sup>20</sup> One reason for the wide range of dopamine affinities at the D<sub>2</sub>High site is that experiments use different levels of NaCl in the buffer. High levels of NaCl convert D<sub>2</sub>High receptors into their D<sub>2</sub>Low state, especially at 37°C,<sup>21</sup> obscuring the dissociation constant of dopamine at D<sub>2</sub>High, and increasing the error in the computer-assisted resolution of the dissociation constant at the high-affinity state. A low concentration of NaCl (10 mM) permits a clear demarcation of the D<sub>2</sub>High state as measured with [<sup>3</sup>H]raclopride, with 40% of the receptors being in the high-affinity state. Thus, experiments to assess the affinity of ketamine and PCP to the high-affinity state were carried out under 10 mM conditions. To confirm that what was being measured was the high-affinity site of the receptor, the binding was carried out with and without 200  $\mu$ M guanilylimidodiphosphate (Gpp[NH]p) in the presence of the endogenous ligand. In each case it was documented that the low-affinity state was unaffected by Gpp[NH]p while the high-affinity state was totally eliminated (Figure 1).

Once the high-affinity state of each of the receptors was confirmed as above, experiments were carried out



**Figure 1** The figures demonstrate the clear delineation of high- and low-affinity states of the dopamine D<sub>2</sub> receptors, under conditions of 10 mM NaCl (b), as confirmed by their disappearance in the presence of 200 μM guanylimidodiphosphate. The high-affinity states are not discernible in the presence of 120 mM NaCl, though the low-affinity states can be reliably detected (a). The final concentration of [<sup>3</sup>H]raclopride was 2 nM, non-specific binding was assessed in the presence of 10 μM S-sulpride.

with ketamine and PCP included in the competition to determine their affinity for the different receptors. This was achieved by adding different concentrations of ketamine (0.1–300 μM) and PCP (0.1–1000 μM) to compete with the radiolabeled ligand ([<sup>3</sup>H]raclopride and [<sup>3</sup>H]ketanserin) for the high-affinity site of the receptor. To confirm the effects on the high-affinity site via direct competition with the endogenous agonist ligand, we examined the effect of ketamine and PCP on the dopamine D<sub>2</sub> receptor high-affinity state of cloned dopamine D<sub>2</sub>long (human) receptors as expressed on CHO cells by competing against 4 nM [<sup>3</sup>H]dopamine.

The competition data were analysed, as previously described.<sup>21</sup> The data were examined to see whether a two-site fit was better than a one-site fit, and if the data conformed better to a two-site fit, the radioligand affinity for each site was determined.

#### [<sup>35</sup>S]GTP-γ-S incorporation

Agonist binding to G protein-coupled receptors leads to a coupling of GTP to the agonist-receptor complex, a process that can be measured using radiolabeled

GTP-γ-S.<sup>22</sup> Gardner and Strange<sup>23</sup> have demonstrated that the stimulation of [<sup>35</sup>S]GTP-γ-S binding in Chinese hamster ovary (CHO) cells expressing recombinant D<sub>2</sub>long receptors provides a valid system for examining the binding and efficacy of agonists. D<sub>2</sub>long receptors were expressed in CHO cells as described before.<sup>18</sup> The ability of ketamine and PCP to stimulate [<sup>35</sup>S]GTP-γ-S incorporation in cells expressing and devoid of D<sub>2</sub> receptors was measured as well as in the presence and absence of a dopamine D<sub>2</sub> antagonist (0.1–100 nM raclopride), along procedures described by Gardner and Strange.<sup>23</sup> Briefly, the cells were suspended in assay buffer (50 mM Tris, pH 7.4, 1 mM EDTA, 5 mM KCl, 4 mM MgCl<sub>2</sub>, 1.5 mM CaCl<sub>2</sub>, 10 mM NaCl and 10 μM GDP) and then transferred to a glass test tube (12 × 75 mm) which received 0.25 ml of the test compound, 0.25 ml of [<sup>35</sup>S]GTP-γ-S (1250 Ci mmol<sup>-1</sup>, ~0.2 nM) and 0.5 ml of cell suspension. The reaction mixtures were incubated for 30 min in a 30°C water bath. The reaction was terminated by rapid filtration and radioactivity measured by liquid scintillation spectrometry.

The affinity of ketamine for dopamine D<sub>2</sub> receptors was reported in a recent letter-to-the-editor in response to an issue germane to this topic.<sup>24</sup>

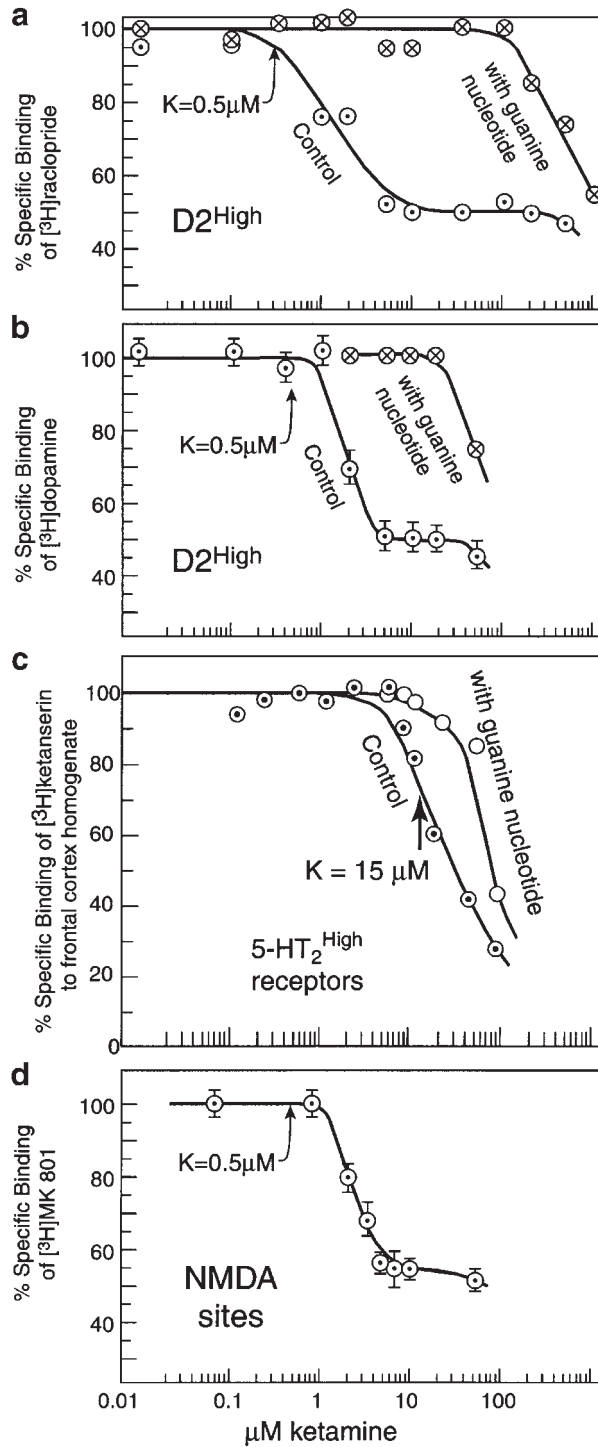
## Results

Under the experimental conditions described we observed a clear demarcation between the high- and low-affinity states of the receptors. The addition of 200 μM Gpp[NH]p showed no effect on the low-affinity states, while the high-affinity states were totally eliminated, thus validating the experimental conditions (see Figure 1, data for the dopamine D<sub>2</sub> receptors shown).

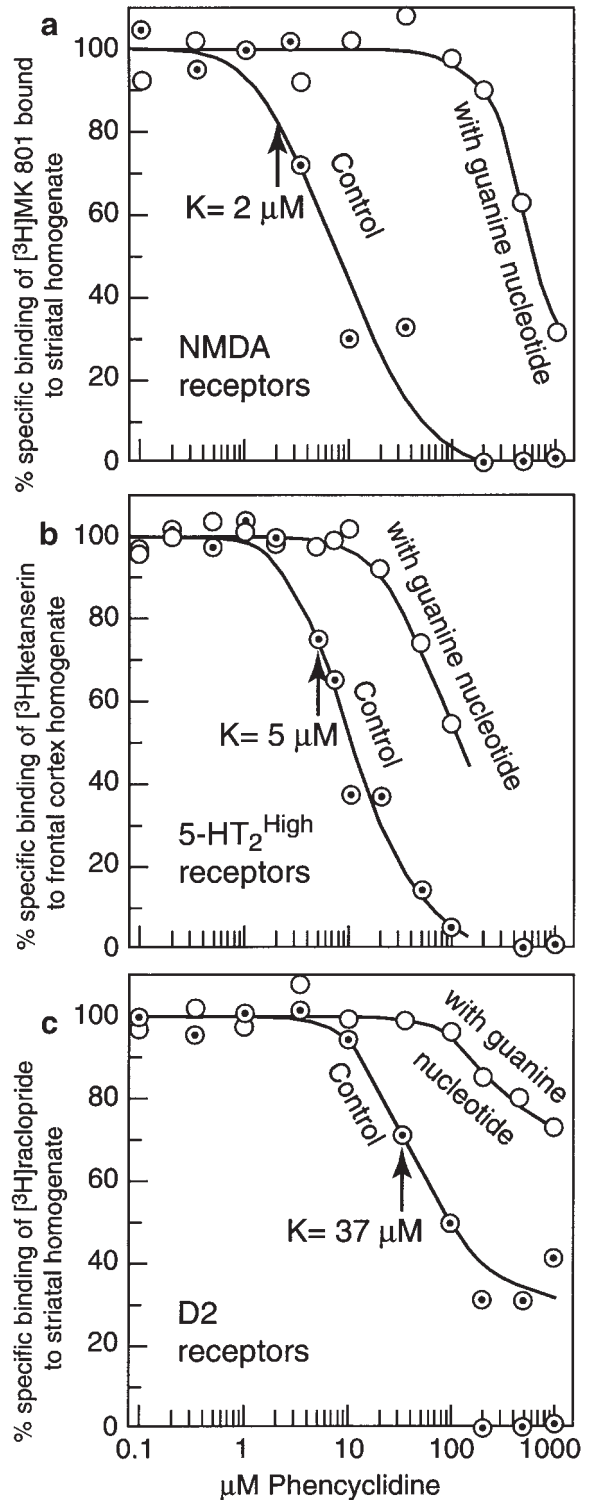
Ketamine had an affinity ( $K_i$  of  $1.0 \pm 0.2$  μM for the dopamine D<sub>2</sub>receptors (using [<sup>3</sup>H]raclopride and striatal tissue) and an affinity of  $0.5 \pm 0.2$  μM (using cloned receptors and [<sup>3</sup>H]-dopamine). This was comparable to its affinity for the NMDA receptors labeled with [<sup>3</sup>H]MK-801 ( $0.5 \pm 0.15$  μM). Ketamine also showed appreciable affinity for the high-affinity state of the serotonin 5-HT<sub>2</sub> receptors ( $15 \pm 5$  μM) (Figure 2). When S-ketamine was compared to its racemic mixture in parallel runs, S-ketamine showed a two- to three-times higher affinity for both the high-state of the dopamine D<sub>2</sub> receptor defined by [<sup>3</sup>H]raclopride ( $0.7 \pm 0.3$  μM vs  $2.3 \pm 0.3$ ) as well as for the NMDA receptor defined by [<sup>3</sup>H]-MK-801 ( $0.5$  μM vs  $1.4$  μM).

PCP showed a similar high-affinity for the high-affinity state of the serotonin 5-HT<sub>2</sub> receptors ( $5 \pm 2$  μM), as it did for the NMDA receptor as labeled with [<sup>3</sup>H]MK-801 ( $2 \pm 0.5$  μM) (Figure 3). And while its affinity for the D<sub>2</sub> receptor was relatively lower ( $37 \pm 10$  μM), it is still high enough to preclude selectivity at clinically relevant concentrations.

When cloned D<sub>2</sub> receptors were exposed to ketamine and PCP they showed a significant, dose-dependent increase in the binding of [<sup>35</sup>S]GTP-γ-S with an EC<sub>50</sub> of  $0.9 \pm 0.4$  μM for ketamine and  $4 \pm 1$  μM for PCP (Figure



**Figure 2** Ketamine had similar affinity for the NMDA receptors (d; rat striatal homogenate; labelled by 2 nM [ $^3$ H]MK 801) and the high-affinity state of dopamine  $D_2$  receptors, as labelled by either 4 nM [ $^3$ H]dopamine (b; cloned  $D_2$  human receptors) or 2 nM [ $^3$ H]raclopride (a; rat striatal homogenate). Ketamine also showed a relatively high affinity for the high-state of the 5-HT $_2$  receptors (c) indicating that ketamine is not selective for NMDA receptors. The addition of 200  $\mu$ M guanylimidodiphosphate converted the high-affinity states of  $D_2$  into low-affinity states which were not sensitive to ketamine in the  $\mu$ M range (a, b). Shown here are representative experiments.



**Figure 3** PCP had similar affinity for the NMDA receptors (a; rat striatal homogenate; labelled by 2 nM [ $^3$ H]MK 801) the high-affinity state of serotonin 5-HT $_2$  receptors as labeled by 0.9 nM [ $^3$ H]ketanserin (b; rat cortex) and the high-affinity state of the dopamine  $D_2$  receptors, as labelled by 2 nM [ $^3$ H]raclopride (c; rat striatal homogenate). The addition of 200  $\mu$ M guanylimidodiphosphate converted the high-affinity states of 5-HT $_2$  and  $D_2$  into low-affinity states which were not sensitive to PCP in the  $\mu$ M range (a, b, c). Shown here are representative experiments.

4). This increase was selective for CHO cells expressing the  $D_2$  receptors and was not seen in cells without  $D_2$  receptors. The increase for ketamine was in the range of 80% of that produced by dopamine, while that of PCP was in the range of 50% of the dopamine effect, suggestive of a partial agonist effect in this functional assay. The addition of raclopride dose-dependently ( $EC_{50}$  2–5 nM) reversed this incorporation induced by ketamine and PCP, consistent with this incorporation being mediated selectively via the  $D_2$  receptor.

## Discussion

The results show that ketamine and PCP show an affinity for dopamine  $D_2$  and serotonin 5-HT<sub>2</sub> receptors which is in the same range as their affinity for the NMDA receptor. Further, ketamine and PCP differentiate between the high-affinity state and the low-affinity state of these receptors, suggesting an agonist-like binding to these sites, a fact supported by their agonist-like action leading to enhanced incorporation of [<sup>35</sup>S]GTP- $\gamma$ -S in cloned CHO cells expressing  $D_2$  receptors. These findings confound the inferences one can draw from the ketamine and PCP-based animal models of schizophrenia and drug discovery. We discuss these implications below.

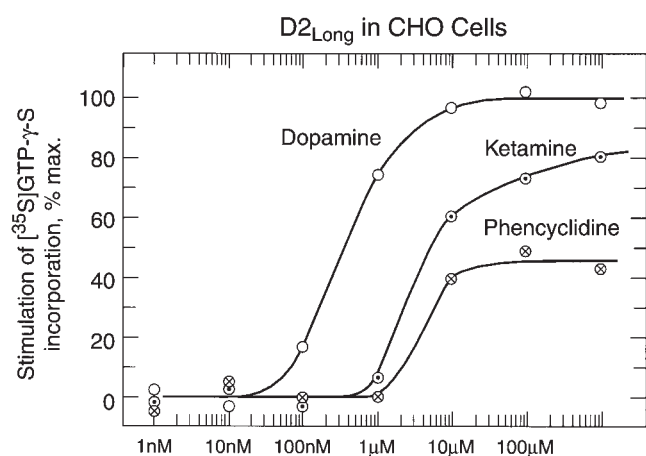
While it has been very well recognized that challenge with ketamine and PCP leads to a broad-based perturbation of neurotransmitter systems,<sup>10,11</sup> it has usually been assumed that these effects were secondary to a primary glutamatergic alteration.<sup>5</sup> However, this specificity is being challenged on several fronts. It is now documented that ketamine has a direct and relevant affinity (47  $\mu$ M) for the dopamine transporter (as measured using cloned receptors and [<sup>3</sup>H]dopamine) and this effect shows stereoselectivity (S- more potent than

R-ketamine).<sup>15,25</sup> Further, it shows relevant affinity (19  $\mu$ M) for the serotonin transporter as assessed using [<sup>3</sup>H]paroxetine.<sup>26,27</sup> Similarly relevant affinities have been shown for PCP at the biogenic amine uptake site, in addition to its affinity for the NMDA-PCP site.<sup>14</sup> The actions are not limited to these systems, and recently it has been shown using recombinant opioid receptors expressed in CHO cells that ketamine shows a stereospecific high-affinity (pK<sub>i</sub> 4.4  $\mu$ M at the  $\mu$  opioid receptor; 4.5  $\mu$ M at the  $\kappa$  opioid receptor and 3.6  $\mu$ M at the  $\delta$  opioid receptor), well within the clinically relevant concentrations.

Despite an extensive search we could not find a study that had systematically examined the effects of ketamine and PCP on dopamine  $D_2$  and serotonin 5-HT<sub>2</sub> receptors. The nearest relevant evidence is a paper by Nabeshima *et al* that showed that PCP *in vitro*, like ritanserin, protected 5-HT<sub>2</sub> receptors from inactivation by sulfhydryl-modifying-agent, N-ethylmaleimide, suggesting that PCP interacts directly with the 5-HT<sub>2</sub> receptor site.<sup>13</sup> It is important to point out that ketamine and PCP exhibited a high (and comparable to NMDA) affinity only for the high-affinity state of the  $D_2$  and 5-HT<sub>2</sub> receptors and showed a much lower affinity (>100  $\mu$ M) for the low-affinity state of these receptors. This ability of ketamine and PCP to differentiate between high- and low-affinity states of the GPCR is characteristic of agonist-like ligands, suggesting that while ketamine and PCP are antagonists at the NMDA receptor site, they may function as agonists at the  $D_2$  and 5-HT<sub>2</sub> site. This finding was confirmed at the  $D_2$  site, by showing that ketamine and PCP led to significant [<sup>35</sup>S]GTP- $\gamma$ -S incorporation, specific to cells expressing the  $D_2$  receptors, and could be competitively blocked by raclopride alone—suggesting that it was a partial agonist effect mediated via dopamine  $D_2$  receptors.

The non-specificity of ketamine and PCP may not be limited to these receptors alone as preliminary data in our laboratory suggest that they have a similarly high-affinity ( $K_i$  in the 1–10  $\mu$ M range) for the high state of the dopamine  $D_1$  receptors and the adrenergic  $\alpha_1$  receptors (unpublished data, further studies in progress).

It is important to note that while the affinities of ketamine and PCP for the dopamine  $D_2$  and serotonin 5-HT<sub>2</sub> are in the  $\mu$ M range, these *are* the functionally relevant concentrations of the use of these drugs in human and animal models. Ketamine has been used extensively as an anesthetic agent and the plasma concentrations associated with subanesthetic psychosis-like perceptual disturbances are in the range of 0.5–1  $\mu$ M,<sup>28</sup> and with anesthesia setting in at concentrations in the 10–100  $\mu$ M range.<sup>29–31</sup> Further, brain concentrations are known to be several fold higher than plasma concentrations,<sup>32</sup> suggesting that even the subanesthetic psychosis-like perceptual disturbances occur only at brain concentrations in the one to few micromolar range. Since the affinities of ketamine for the NMDA receptors as well as for the dopamine  $D_2$  and serotonin 5-HT<sub>2</sub> are relevant in this  $\mu$ M range, this



**Figure 4** Ketamine and PCP enhanced the incorporation of [<sup>35</sup>S]GTP- $\gamma$ -S into  $D_2$  long-transfected cells. The maximal effects of ketamine and PCP were lower than that of dopamine, suggesting a partial agonist effect. The  $EC_{50}$  for the agonist incorporation are consistent with their  $K_i$  values at the  $D_2$  high-affinity states and this induction was completely inhibited by the addition of raclopride, in a dose-dependent manner. Shown here are representative experiments.

would make a selective disruption of NMDA transmission by these agents unlikely.

This nonspecificity of ketamine and PCP poses some challenges for the use of these agents as 'models' of schizophrenia. Ketamine and PCP administration in humans lead to perceptual and cognitive abnormalities and schizophrenia-like disturbances reminiscent of the 'positive' and 'negative' symptoms of schizophrenia.<sup>8</sup> It is commonly argued that the similarity between the drug-induced state and schizophrenia provides some evidence for a 'primary hypoglutamate' state in schizophrenia.<sup>8,33,34</sup> However, at the concentrations employed in human models, these drugs are not only likely to have an effect on the NMDA receptor system, but are also likely to have relevant effects on several G-protein coupled-receptors (notably D<sub>2</sub> and 5-HT<sub>2</sub> as well as monoamine transporters<sup>15,25</sup> and the opioid receptor system.<sup>35</sup> In fact, more recent studies suggest that ketamine and PCP lead to a state of increased, as opposed to decreased, glutamatergic transmission further calling into question the use of these agents as models of the 'hypoglutamate' hypothesis.<sup>36,37</sup>

Ketamine and PCP are more commonly used in animal models to produce a state of hyperlocomotion or disruption of sensorimotor gating. This altered state is more effectively reversed by the administration of 'atypical' rather than 'typical' antipsychotics. This has led to the suggestion that these models, by recapitulating the hypoglutamate state of schizophrenia, are a better model to identify 'atypical' antipsychotics.<sup>38</sup> There are several caveats to this line of reasoning. First, the claim that ketamine/PCP models accurately distinguish typical from atypical antipsychotic drugs is not always substantiated in animal studies as several experiments show that typical antipsychotics can equally well block the effects of ketamine and PCP.<sup>39,40</sup> More importantly, studies in patients with PCP-induced psychosis (the state that the animal models are presumably reflecting) show that typical antipsychotics such as haloperidol and pimozide (a selective D<sub>2/3</sub> blocker) are much more effective than placebo, and result in remission of all symptoms.<sup>41-43</sup> To our knowledge there are no data on the use of atypical antipsychotics in clinical PCP-induced psychosis.

Our finding would suggest that ketamine and PCP (especially as compared to the more conventional apomorphine/amphetamine model) would likely lead to a more widespread and non-specific neurochemical perturbation which would encompass direct and indirect changes in the glutamate, dopaminergic and serotonergic systems. Thus, one would expect that drugs which have a more widespread (and non-specific) pharmacology would be more effective in this model, regardless of whether they are 'typical' or 'atypical'; and for that matter regardless of whether they are antipsychotic or not. This seems to be the case. Chlorpromazine (a typical antipsychotic with broad-spectrum neurotransmitter actions including anti-serotonin effects) has been shown to be as effective as clozapine and olanzapine (atypical antipsychotics with several actions including anti-serotonin effects).<sup>39</sup> Con-

versely, amisulpiride (an atypical antipsychotic with no action on the serotonin system) is ineffective in these models,<sup>44</sup> even though it is clinically an 'atypical' antipsychotic.<sup>45</sup>

Consistent with our findings, a recent analysis suggests that activity in ketamine/PCP vs amphetamine/apomorphine models segregates largely along 5-HT<sub>2</sub> vs D<sub>2</sub> lines: drugs with a potent action on the serotonin 5-HT<sub>2</sub> system being more effective in the PCP model;<sup>44,46</sup> while drugs with a prominent action on the D<sub>2</sub> receptor being more effective in the apomorphine/amphetamine models;<sup>44</sup> and drugs with a mixed action being effective in both<sup>44</sup> regardless of whether these drugs are antipsychotic or not.<sup>44</sup> For example, fananserin (a 5-HT<sub>2</sub>/D<sub>4</sub> antagonist), MDL 100,907 (a selective 5-HT<sub>2</sub> antagonist), prazosin (a selective adrenergic  $\alpha_1$  antagonist) are effective in the ketamine/PCP models<sup>44,47-50</sup> even though these drugs fail to show antipsychotic activity in the clinic.<sup>51,52</sup>

Several limitations of our findings and their implications need to be stated. The study is based on the affinity of ketamine and PCP for the *high-affinity state* of the D<sub>2</sub> and 5-HT<sub>2</sub> receptors, as it is this state of the receptor that is thought to be functionally important.<sup>16</sup> However, the high-affinity states are sensitive to experimental conditions,<sup>21</sup> therefore, the implications hold only insofar as the *in vitro* measurements reflect the status of high states *in vivo*. Secondly, our data should not be read to question the direct effect of ketamine and PCP as non-competitive NMDA receptor antagonists. This is a well established point and consistent with this direct effect, allosteric modifications of the NMDA receptor can reverse some actions of PCP in animal models.<sup>53</sup> Thus, we do not question the direct effect, just its selectivity at relevant doses. Third, while we have shown a direct effect on the dopamine D<sub>2</sub> and serotonin 5-HT<sub>2</sub> receptors *in vitro*, it is yet to be determined which of the behavioural effects of these challenges are attributable to these direct effects. It should be mentioned that at present the data on this issue are conflicting. Some aspects of these challenges can be reversed by D<sub>2</sub> blockers,<sup>40,54-56</sup> some by 5-HT<sub>2</sub> blockers,<sup>57-59</sup> and some are not.<sup>56</sup> Finally, even if ketamine and PCP are non-specific *in vivo*, it does *not* invalidate the ketamine/PCP as models for studying schizophrenia. It could well be that schizophrenia itself is a multi-transmitter dysfunction and ketamine and PCP, by virtue of their relatively broad-based neurotransmitter perturbation, provide a better model of the complexity of this illness than a primary dopaminergic or a primary hypoglutamate model. Whether this is indeed the case cannot be unequivocally decided until the true nature of the neurochemical dysfunction in schizophrenia is delineated.

In summary, the data in this study suggest that ketamine and PCP, in addition to being non-competitive NMDA receptor antagonists, have a high-affinity for the dopamine D<sub>2</sub> and the serotonin 5-HT<sub>2</sub> site and act as partial agonists at the D<sub>2</sub> receptor. These findings question the inferences that can be drawn from ketamine and PCP challenges in animal models and patients.

More selective NMDA receptor antagonists are needed to advance the study of the role of the glutamate system in schizophrenia and antipsychotic action.

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### References

- 1 Laruelle M, Abi-Dargham A. Dopamine as the wind of the psychotic fire: new evidence from brain imaging studies. *J Psychopharmacol* 1999; **13**: 358–371.
- 2 Seeman P, Kapur S. Schizophrenia: more dopamine, more D2 receptors. *Proc Natl Acad Sci U S A* 2000; **97**: 7673–7675.
- 3 Kapur S, Seeman P. Does fast dissociation from the dopamine D2 receptors explain atypical antipsychotic action—a new hypothesis. *Am J Psychiatry* 2001; **158**: 360–369.
- 4 Kim JS, Kornhuber HH, Schmid-Burgk W, Holzmüller B. Low cerebrospinal fluid glutamate in schizophrenic patients and a new hypothesis on schizophrenia. *Neurosci Lett* 1980; **20**: 379–382.
- 5 Carlsson M, Carlsson A. Interactions between glutamatergic and monoaminergic systems within the basal ganglia—implications for schizophrenia and Parkinson's disease. *Trends Neurosci* 1990; **13**: 272–276.
- 6 Carlsson A, Waters N, Waters S, Carlsson ML. Network interactions in schizophrenia—therapeutic implications. *Brain Res Brain Res Rev* 2000; **31**: 342–349.
- 7 Javitt DC. Glutamate receptors and schizophrenia: opportunities and caveats. *Mol Psychiatry* 1996; **1**: 16–17.
- 8 Jentsch JD, Roth RH. The neuropsychopharmacology of phencyclidine: from NMDA receptor hypofunction to the dopamine hypothesis of schizophrenia. *Neuropsychopharmacology* 1999; **20**: 201–225.
- 9 Swerdlow NR, Braff DL, Bakshi VP, Geyer MA. An animal model of sensorimotor gating deficits in schizophrenia predicts antipsychotic drug action. In: Csernansky JG (ed). *Antipsychotics*. Heidelberg Platz 3/W-1000 Berlin 33/Germany: Springer-Verlag; Berlin, 1996, pp 289–312.
- 10 Kari HP, Davidson PP, Kohl HH, Kochhar MM. Effects of ketamine on brain monoamine levels in rats. *Res Commun Chem Pathol Pharmacol* 1978; **20**: 475–488.
- 11 Glisson SN, el-Etr AA, Bloor BC. The effect of ketamine upon norepinephrine and dopamine levels in rabbit brain parts. *Naunyn Schmiedeberg's Arch Pharmacol* 1976; **295**: 149–152.
- 12 Tsukada H, Harada N, Nishiyama S, Ohba H, Sato K, Fukumoto D *et al*. Ketamine decreased striatal [(11)C]raclopride binding with no alterations in static dopamine concentrations in the striatal extracellular fluid in the monkey brain: multiparametric PET studies combined with microdialysis analysis. *Synapse* 2000; **37**: 95–103.
- 13 Nabeshima T, Ishikawa K, Yamaguchi K, Furukawa H, Kameyama T. Protection with phencyclidine against inactivation of 5-HT<sub>2</sub> receptors by sulfhydryl-modifying reagents. *Biochem Pharmacol* 1988; **37**: 3277–3283.
- 14 Rothman RB. PCP site 2: a high affinity MK-801-insensitive phencyclidine binding site. *Neurotoxicol Teratol* 1994; **16**: 343–353.
- 15 Nishimura M, Sato K, Okada T, Yoshiya I, Schloss P, Shimada S *et al*. Ketamine inhibits monoamine transporters expressed in human embryonic kidney 293 cells. *Anesthesiology* 1998; **88**: 768–774.
- 16 George SR, Watanabe M, Di Paolo T, Falardeau P, Labrie F, Seeman P. The functional state of the dopamine receptor in the anterior pituitary is in the high affinity form. *Endocrinology* 1985; **117**: 690–697.
- 17 Seeman P, Ulpian C, Wreggett KA, Wells JW. Dopamine receptor parameters detected by [3H]spiperone depend on tissue concentration: analysis and examples. *J Neurochem* 1984; **43**: 221–235.
- 18 Liu IS, George SR, Seeman P. The human dopamine D<sub>2</sub>(Longer) receptor has a high-affinity state and inhibits adenylyl cyclase. *Brain Res Mol Brain Res* 2000; **77**: 281–284.
- 19 Malmberg A, Mohell N. Characterization of [3H]quinpirole binding to human dopamine D<sub>2A</sub> and D<sub>3</sub> receptors: effects of ions and guanine nucleotides. *J Pharmacol Exp Ther* 1995; **274**: 790–797.
- 20 MacKenzie RG, VanLeeuwen D, Pugsley TA, Shih YH, Demattos S, Tang L *et al*. Characterization of the human dopamine D<sub>3</sub> receptor expressed in transfected cell lines. *Eur J Pharmacol* 1994; **266**: 79–85.
- 21 Watanabe M, George SR, Seeman P. Dependence of dopamine receptor conversion from agonist high- to low-affinity state on temperature and sodium ions. *Biochem Pharmacol* 1985; **34**: 2459–2463.
- 22 Lefkowitz RJ, Cotecchia S, Samama P, Costa T. Constitutive activity of receptors coupled to guanine nucleotide regulatory proteins. *Trends Pharmacol Sci* 1993; **14**: 303–307.
- 23 Gardner B, Strange PG. Agonist action at D<sub>2</sub>(long) dopamine receptors: ligand binding and functional assays. *Br J Pharmacol* 1998; **124**: 978–984.
- 24 Kapur S, Seeman P. Ketamine has equal affinity for NMDA receptors and the high-affinity state of the dopamine D<sub>2</sub> receptor. *Biol Psychiatry* 2001; **49**: 954–957.
- 25 Nishimura M, Sato K. Ketamine stereoselectively inhibits rat dopamine transporter. *Neurosci Lett* 1999; **274**: 131–134.
- 26 Martin DC, Introna RP, Aronstam RS. Inhibition of neuronal 5-HT uptake by ketamine, but not halothane, involves disruption of substrate recognition by the transporter. *Neurosci Lett* 1990; **112**: 99–103.
- 27 Martin DC, Adams RJ, Watkins CA. Inhibition of synaptosomal serotonin uptake by Ketalar. *Res Commun Chem Pathol Pharmacol* 1988; **62**: 129–132.
- 28 Bowdle TA, Radant AD, Cowley DS, Kharasch ED, Strassman RJ, Roy-Byrne PP. Psychedelic effects of ketamine in healthy volunteers: relationship to steady-state plasma concentrations. *Anesthesiology* 1998; **88**: 82–88.
- 29 Geisslinger G, Hering W, Thomann P, Knoll R, Kamp HD, Brune K. Pharmacokinetics and pharmacodynamics of ketamine enantiomers in surgical patients using a stereoselective analytical method. *Br J Anaesth* 1993; **70**: 666–671.
- 30 Domino EF, Zsigmond EK, Domino LE, Domino KE, Kothary SP, Domino SE. Plasma levels of ketamine and two of its metabolites in surgical patients using a gas chromatographic mass fragmentographic assay. *Anesth Analg* 1982; **61**: 87–92.
- 31 Idvall J, Ahlgren I, Aronsen KR, Stenberg P. Ketamine infusions: pharmacokinetics and clinical effects. *Br J Anaesth* 1979; **51**: 1167–1173.
- 32 Shimoyama M, Shimoyama N, Gorman AL, Elliott KJ, Inturrisi CE. Oral ketamine is antinociceptive in the rat formalin test: role of the metabolite, norketamine. *Pain* 1999; **81**: 85–93.
- 33 Tamminga CA. Schizophrenia and glutamatergic transmission. *Crit Rev Neurobiol* 1998; **12**: 21–36.
- 34 Carlsson M, Carlsson A. Schizophrenia: a subcortical neurotransmitter imbalance syndrome? [Review]. *Schizophr Bull* 1990; **16**: 425–432.
- 35 Hirota K, Okawa H, Appadu BL, Grandy DK, Devi LA, Lambert DG. Stereoselective interaction of ketamine with recombinant mu, kappa, and delta opioid receptors expressed in Chinese hamster ovary cells. *Anesthesiology* 1999; **90**: 174–182.
- 36 Moghaddam B, Adams BW. Reversal of phencyclidine effects by a group II metabotropic glutamate receptor agonist in rats. *Science* 1998; **281**: 1349–1352.
- 37 Moghaddam B, Adams B, Verma A, Daly D. Activation of glutamatergic neurotransmission by ketamine: a novel step in the pathway from NMDA receptor blockade to dopaminergic and cognitive disruptions associated with the prefrontal cortex. *J Neurosci* 1997; **17**: 2921–2927.

- 38 Swerdlow NR, Geyer MA. Using an animal model of deficient sensorimotor gating to study the pathophysiology and new treatments of schizophrenia. *Schizophr Bull* 1998; **24**: 285–301.
- 39 Swerdlow NR, Bakshi V, Waikar M, Taaid N, Geyer MA. Seroquel, clozapine and chlorpromazine restore sensorimotor gating in ketamine-treated rats. *Psychopharmacol (Berl)* 1998; **140**: 75–80.
- 40 Verma A, Kulkarni SK. Modulation of MK-801 response by dopaminergic agents in mice. *Psychopharmacology* 1992; **107**: 431–436.
- 41 Giannini AJ, Eighan MS, Loiselle RH, Giannini MC. Comparison of haloperidol and chlorpromazine in the treatment of phencyclidine psychosis. *J Clin Pharmacol* 1984; **24**: 202–204.
- 42 Giannini AJ, Nageotte C, Loiselle RH, Malone DA, Price WA. Comparison of chlorpromazine, haloperidol and pimozide in the treatment of phencyclidine psychosis: DA-2 receptor specificity. *J Toxicol Clin Toxicol* 1984; **22**: 573–579.
- 43 Giannini AJ, Price WA, Loiselle RH, Malone DW. Treatment of phenylcyclohexylpyrrolidine (PHP) psychosis with haloperidol. *J Toxicol Clin Toxicol* 1985; **23**: 185–189.
- 44 Millan MJ, Brocco M, Gobert A, Joly F, Bervoets K, Rivet J *et al*. Contrasting mechanisms of action and sensitivity to antipsychotics of phencyclidine versus amphetamine: importance of nucleus accumbens 5-HT<sub>2A</sub> sites for PCP-induced locomotion in the rat. *Eur J Neurosci* 1999; **11**: 4419–4432.
- 45 Geddes J, Freemantle N, Harrison P, Bebbington P. Atypical antipsychotics in the treatment of schizophrenia: systematic overview and meta-regression analysis. *BMJ* 2000; **321**: 1371–1376.
- 46 Yamada S, Harano M, Annoh N, Nakamura K, Tanaka M. Involvement of serotonin 2A receptors in phencyclidine-induced disruption of prepulse inhibition of the acoustic startle in rats. *Biol Psychiatry* 1999; **46**: 832–838.
- 47 Carlsson ML. The selective 5-HT<sub>2a</sub> receptor antagonist MDL 100,907 counteracts the psychomotor stimulation ensuing manipulations with monoaminergic, glutamatergic or muscarinic neurotransmission in the mouse—implications for psychosis. *J Neural Transm—Gen Sect* 1995; **100**: 225–237.
- 48 Arvanov VL, Wang RY. M100907, a selective 5-HT<sub>2A</sub> receptor antagonist and a potential antipsychotic drug, facilitates N-methyl-D-aspartate-receptor mediated neurotransmission in the rat medial prefrontal cortical neurons in vitro. *Neuropsychopharmacology* 1998; **18**: 197–209.
- 49 Wang RY, Liang X. M100907 and clozapine, but not haloperidol or raclopride, prevent phencyclidine-induced blockade of NMDA responses in pyramidal neurons of the rat medial prefrontal cortical slice. *Neuropsychopharmacology* 1998; **19**: 74–85.
- 50 Bakshi VP, Geyer MA. Phencyclidine-induced deficits in prepulse inhibition of startle are blocked by prazosin, an alpha-1 noradrenergic antagonist. *J Pharmacol Exp Ther* 1997; **283**: 666–674.
- 51 Announcement. Management decisions on priority pipeline products—MDL 100907. In: *Vision Extra*. 1999, pp 2–3.
- 52 Truffinet P, Tamminga CA, Fabre LF, Meltzer HY, Riviere ME, Papillon-Downey C. Placebo-controlled study of the D<sub>4</sub>/5-HT<sub>2A</sub> antagonist fananserin in the treatment of schizophrenia. *Am J Psychiatry* 1999; **156**: 419–425.
- 53 Toth E, Lajtha A. Antagonism of phencyclidine-induced hyperactivity by glycine in mice. *Neurochem Res* 1986; **11**: 393–400.
- 54 Kuribara H, Uchihashi Y. SCH 23390 equivalently, but YM-09151-2 differentially reduces the stimulant effects of methamphetamine, MK-801 and ketamine: assessment by discrete shuttle avoidance in mice. *Jpn J Pharmacol* 1993; **62**: 111–114.
- 55 Jackson DM, Johansson C, Lindgren LM, Bengtsson A. Dopamine receptor antagonists block amphetamine and phencyclidine-induced motor stimulation in rats. *Pharmacol Biochem Behav* 1994; **48**: 465–471.
- 56 Krystal JH, D'Souza DC, Karper LP, Bennett A, Abi-Dargham A, Abi-Saab D *et al*. Interactive effects of subanesthetic ketamine and haloperidol in healthy humans. *Psychopharmacol (Berl)* 1999; **145**: 193–204.
- 57 Nabeshima T, Ishikawa K, Yamaguchi K, Furukawa H, Kameyama T. Phencyclidine-induced head-twitch responses as 5-HT<sub>2</sub> receptor-mediated behavior in rats. *Neurosci Lett* 1987; **76**: 335–338.
- 58 Varty GB, Bakshi VP, Geyer MA. M100907, a serotonin 5-HT<sub>2A</sub> receptor antagonist and putative antipsychotic, blocks dizocilpine-induced prepulse inhibition deficits in Sprague-Dawley and Wistar rats. *Neuropsychopharmacology* 1999; **20**: 311–321.
- 59 Wang RY, Liang X. M100907 and clozapine, but not haloperidol or raclopride, prevent phencyclidine-induced blockade of NMDA responses in pyramidal neurons of the rat medial prefrontal cortical slice. *Neuropsychopharmacology* 1998; **19**: 74–85.